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of the viruses detected in the samples. However, the sequencing method has the limitation that it cannot be applied in clinical laboratories.

Aim of the study: Design a test kit using multiplex real-time PCR that can be performed in diagnostic laboratories to detect 4 variants Alpha, Beta, Gamma and Delta and two mutations that help the virus to spread rapidly (D614G) and can escape the action of specific antibodies (E484 K).

Material and method: Primers and probes to detect Alpha, Beta, Gamma and Delta variants are designed based on the detection of specific mutations of these variants. The Alpha, Beta and Delta variants were detected based on ARMS Taqman real-time PCR (ARMS: Amplification Refractory Mutation System) with the principle that if a mutation is present, the Taqman probe will not be hydrolyzed and will not have an amplified signal, if there is no mutation the Taqman probe will be hydrolyzed and will have an amplified signal. The Gamma variant and the D614G mutations as well as the E484 K mutations were detected based on the SNP Taqman real-time PCR with the principle that each mutation would be detected by two Taqman probes with different reporters, FAM and HEX or TexasRED and CY5 and depending on the early or late of the fluorescent signal of these two Taqman probes, it can be concluded whether or not there is a mutation. The test kit is designed with three RT multiplex real-time PCR with multiplex A (MPL-A) to detect SARS-CoV-2 based on E gene using primers and Taqman probe (FAM) according to WHO design, variant Alpha (HEX) and the internal control is the RNaseP gene (CY5); MPL-B detects Delta variant (FAM), Beta variant (HEX), and Gamma variant (TexasRED/CY5); MPL-C detects D614G (FAM/HEX) and E484 K (TexasRED/CY5). The multiplex was prepared from AgPath-ID™ One-Step RT-PCR (ThermoFisher, USA). To check the primers and probes, the corresponding DNA sequences for the mutants were also designed as controls [+]. The test kit is then tested on samples that are the RNA extracts positive with SARS-CoV-2.

Results: Testing on [+] controls showed that the detection limit for the E gene and the Alpha variant was 10-6 fm/μl, the Delta variant was 10-5 fm/μl, and the Beta and Gamma variant was 10-7 fm/μl, the D614G and E484G mutations were 10-5 fm/μl. There was no cross-detection of mutations or variants. Tested on RNA extracts that were positive with SARS-CoV-2, the results said that: In HCMC, the strain (1) taken in April 2020 is the wild type, while all strains (12) taken in June 2021 are Delta variants with additional mutations D614G and no mutations E484 K; In Quang Nam, the samples taken in June 2020 are both wild type (2) and have mutation D614G (3), while in June 2021 all strains were variants Alpha (4) and has the D614G mutation. The sample with the wild type, with the Delta variant, with the Alpha variant and the sample with only the D614G mutation were sequenced the whole S gene and the results were completely consistent with the real-time PCR results.

Conclusion: According to the laws of evolution, a rapidly spreading variant will gradually replace the original wild strain, and once community immunity to a variant is achieved, it may be susceptible to another variant and it can therefore replace the old one. Therefore, it is necessary to develop and set-up the multiplex real-time PCR test to detect 4 rapid transmission variants in diagnostic laboratories. With the collected results on the stock samples, we can conclude that at the beginning of the epidemic in Ho Chi Minh City, SARS-CoV-2 was still the original wild strain, but now it has been completely replaced by the Delta variant. In Quang Nam, the beginning of the epidemic was a wild strain but also circulating a strain with a D614G mutation, however the Alpha variant is currently circulating and has a D614G mutation. Particularly, the E484 K mutation has not appeared so far and this is an indication that SARS-CoV-2 has not yet been resistant to specific antibodies that recognize the receptor on the spike protein of the virus.

Keywords: SARS-CoV-2 variants, RT Multiplex real-time PCR.

PCO-011

Assessment of serial monitoring of inflammatory markers in hospital in-patients

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Background: Inflammatory markers such as C-Reactive Protein (CRP) and D-dimer have played a key role in prognostication, triaging of COVID-19 patients. The Ministry of Health and Family Welfare (MoHFW) India has proposed national guidance on the serial monitoring of inflammatory markers as a part of management for hospitalized COVID patients.

Objectives: We aimed to review if the serial monitoring of inflammatory markers adheres to existing national guidance.

Methods: A retrospective review of electronic patient records of 100 hospital in-patients with swab-confirmed COVID-19 was conducted as a baseline audit in which documentation on serial monitoring with CRP and D-dimer on days 1, 4, 7, 10 was checked for patients with moderate to severe COVID disease. Multiple improvement strategies were subsequently implemented and assessed via Plan-Do-Study-Act (PDSA) cycles. A need for more consistent monitoring of markers was emphasized to the treating faculty mainly in form of departmental meetings, in-house clinical seminars. Repeat survey was carried out after a gap of 4 weeks.

Results: Baseline audit highlighted two components were deemed essential: (1) Baseline record of the markers which is up to 4 days of admission; (2) Final record of the markers which is within a period of 4 days prior to discharge. The frequency of these components saw significant improvement by completion of the final PDSA cycle.

Conclusion: The serial monitoring of inflammatory markers did not fall within the existing national guidance. There is a scope for larger studies to validate the serial use of markers, cost benefit and utility of these tests and to determine the frequency of their repeatability in terms of difference it can make in terms of disease outcome.

Keywords: COVID-19, inflammatory markers.

PCO-012

Place of COVID-19 transmission in blood transmission: A case report

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A novel human coronavirus, SARS-CoV-2, has emerged from China in December 2019. It has spread worldwide, conforming to person-to-person transmission. Though the studies have found 15% to 40% of symptomatic patients had detectable RNA-emia, it is not known whether COVID-19 may be transmitted by blood transfusion. Also, according to the worldwide data, only less than 10% do annual blood donations. As Blood and blood components are essential inpatient management it is very important to know whether the SARS-CoV-2 virus is transfusion-transmitted.

Case report: A 33-year-old had donated a whole blood unit at a mobile blood donation campaign on 08/12/2020. The donor was healthy, asymptomatic, and no evidence to suspect COVID-19 infection at the time of donation. He had completed the routine pre-donation procedures including screening questionnaire, temperature check, and short medical review. It concluded without any post-donation complications.

On 19/12/2020, the donor was identified as a COVID-19 confirmed case. 11 days after the contact, all first contacts related to the blood transfusion process, remaining Fresh Frozen Plasma pack, and the

recipient of red cell unit tested for COVID-19 PCR and all were non-reactive. None of the recipients developed any COVID-19 related symptoms post-transfusion

Conclusion: None of the recipients of donor diagnosed with COVID-19 following donation developed COVID-19 related symptoms or tested positive for COVID-19 PCR and remaining blood products were also negative for COVID-19 PCR in this asymptomatic blood donor. Continuous data collection is needed to conclude the possibility of COVID-19 transmission through blood transfusion.

PCO-013

Vaccine-associated disease enhancement: a case report of post-vaccination COVID-19

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Introduction: The COVID-19 pandemic has entered a new phase with the roll-out of several vaccines worldwide at an accelerated phase. Currently, little is known about the potential of vaccine associated disease enhancement (VADE) following COVID-19 immunization.

Case Illustration: We herewith report two patients admitted with confirmed COVID-19 pneumonia with a history of CoronaVac vaccination. The first patient with a relatively milder course of the disease had received two doses of CoronaVac whereas the second patient with a more progressive course of the disease received only one dose before developing symptoms and being admitted to the hospital. Our observations suggest that vaccination could act in boosting the inflammatory process and reveal the previously asymptomatic COVID-19 illness. Theoretically, vaccines could induce VADE, where only suboptimal, non-protective, titers of neutralizing antibodies were produced or pro-inflammatory T helper type 2 response were induced. Secondly, enhanced respiratory disease (ERD) could manifest, where paradoxically, pulmonary symptoms are more severe due to peribronchial monocytic and eosinophilic infiltration can happen during infection after vaccination or previous infection.

Conclusion: We report two cases of patients developing COVID-19 shortly after vaccination with CoronaVac in which VADE is likely. We recommend that current vaccination strategies consider measurement of neutralizing antibody titer as a guide in ensuring the safest strategy for mass immunization. Studies are needed to investigate the true incidence of VADE on vaccinated individuals and on how to differentiate between severe disease unrelated to vaccination and VADE.

PCO-014

Parenteral and oral anticoagulant treatment for hospitalized and post-discharge patients with COVID-19: A systematic review

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Introduction: Coagulation abnormalities are key features of COVID-19 patients and anticoagulants has been endorsed by different thrombosis and hematological societies. Regardless of the recommendations above, evidence on the benefit and risk of both prophylactic and treatment dose anticoagulants in COVID-19 patients are lacking. This study aims to investigate the literature on oral and parenteral anticoagulants treatment for hospitalized and post-discharge patients with COVID-19.

Methods: Systematic search and handsearching was conducted between 22 November and 9 December 2020 in the following databases: Cochrane, EBSCO, Pubmed, and EMBASE. The inclusion criteria are human study, aged 18 years or older, full-text, English, randomized control trial, meta-analysis, systematic review, and observational study.

Results: The search yield 18 studies on in-hospital anticoagulant use and 2 studies with prior anticoagulant use. Four studies were eligible for quantitative analysis. Three case series were on drugs with anticoagulation effects were eligible for appraisal. None of studies are clinical trial. All studies included were high quality studies based on the Newcastle Ottawa Scale.

Discussion: In the absence of clinical trial results, early findings from the studies in this systematic review demonstrate the benefit of anticoagulation in COVID-19 patients, especially in the setting of increased VTE in patients with severe disease. Surprisingly, to date we found no published studies reporting the use of anticoagulants in COVID-19 patients post-discharge.

Keywords: COVID-19, anticoagulant, VTE, thromboprophylaxis.

PCO-015

Human airway epithelial Calu-3 cells as the potential platform to study the pathophysiology of SARS-CoV-2 isolated in Malaysia

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Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been identified as the etiologic agent for the Coronavirus Disease 2019 (COVID-19) outbreak that started in early December 2019. To date, COVID-19 has caused almost 6000 deaths in Malaysia since its first outbreak in January 2020.

Objective: Understanding the pathophysiology of the virus is important for the researchers to identify the potential targets against COVID-19. For this purpose, the virus must be isolated and propagated in a suitable host that allows the virus to grow well and at the same time would not cause immediate cell death to the host.

Method: In the effort to identify the best host cells for the propagation of SARS-CoV-2, we infected several mammalian cells lines (i.e., Vero, Vero E6, Calu-3, MRC-5, and A549) with different lineages of SARS-CoV-2 that are widely circulated in Malaysia.

Results: We found that SARS-CoV-2 multiplied only in Vero, Vero E6 and Calu-3 cells. Propagation of the virus in these cell lines were